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Towards the characterization of short-term memory of zebrafish: Effect of fixed versus random reward location

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ABSTRACT

The zebrafish has been proposed as an efficient tool for the analysis of behavioral and neurobiological mechanisms of learning and memory. However, compared to traditional laboratory rodents, it is a relatively newcomer. In fact, only limited information on its mnemonic and cognitive abilities has been obtained, and only a small number of learning and memory paradigms have been available for its testing. Previously, we have shown that zebrafish are capable of learning the systematic alternating sequence of reward location in a shuttle box task in which we evaluated behavioral responses manually. Here, we employ a computerized, automated version of this task. We study whether zebrafish can remember the prior location of a reward (the sight of conspecifics) when the location is fixed (constant), or when the sequence of the location of presentation randomly changes between the left and the right side of the experimental tank. We also analyze performance features including the swim speed of experimental fish as well as the temporal changes of the position of fish when the reward (stimulus) is not presented. Our results show that under both the fixed and randomly changing reward location conditions zebrafish exhibit a significant preference for the prior location of reward, albeit the preference is stronger under the fixed location condition. We conclude that adult zebrafish have short-term associative memory that can be induced and quantified in an automated manner.

1. Introduction

Learning and memory are expected to be influenced by a large number of molecular mechanisms only a fraction of which has been discovered (Sweatt, 2009). Systematic, comprehensive and unbiased mutation screening, a forward genetic approach, has been proposed but not yet conducted to address this need (for a review see Gerlai, 2010, 2011). The zebrafish has gained popularity in numerous fields of biology perhaps because this small and prolific vertebrate has turned out to be particularly amenable to high throughput screening (for reviews see Kalueff et al., 2014; Gerlai, 2010). However, the foundation of such screens for the analysis of learning and memory is not yet solid because the number of behavioral test paradigms one could use for screening is small and our understanding of the mnemonic and cognitive features of this species is limited (Gerlai, 2010, 2011).

Nevertheless, the past decade has seen a rapid increase of learning paradigms specifically developed for the zebrafish and these tasks have demonstrated that this little vertebrate has the ability to associate a variety of unconditioned and conditioned stimuli (Fernandes et al., 2014; Morin et al., 2013; Sison and Gerlai, 2011; Zala and Määttänen, 2013), can learn the spatial location of reinforcers (Karnik and Gerlai, 2012; Spence et al., 2011), and can perform well in latent learning tasks (Gómez-Laplaza and Gerlai, 2010). In addition, associative short-term (Jia et al., 2014) as well as long-term memory (Lucon-Xiccato and Dadda, 2014) have also been recently demonstrated in adult zebrafish. Furthermore, the ontogenesis of cognitive and mnemonic abilities of the zebrafish has also been investigated using both classical and operant learning tasks (Valente et al., 2012).

The majority of associative or operant conditioning tasks developed for biomedical model organisms, including the zebrafish, are time consuming as the tasks often require several training trails. Furthermore, these paradigms frequently necessitate the administration of habituation trials prior to actual training (see Fernandes et al., 2014; Sison and Gerlai, 2011 for examples) thereby extending the time to run the task. Nevertheless, a number of learning paradigms developed for the adult zebrafish have already shown great promise for high throughput screening either because the task is fast or/and because it can be run in an automated and thus massively parallel manner (Lucon-Xiccato and Dadda, 2014; Pather and Gerlai, 2009; Jia et al., 2014; Hicks et al., 2006, also see Gerlai, 2011 for review). One of these paradigms is a shuttle box in which the experimental subject is presented with a rewarding (appetitive) visual stimulus on a computer screen (the sight of conspecifics) (Pather and Gerlai, 2009). In this task, the experimental subject

Abbreviations: Inter-stimulus Interval, ISI.

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will need to learn where this reward has just been shown and where it is going to be shown in subsequent presentations (Pather and Gerlai, 2009). By systematically alternating the presentation sequence in the shuttle box, we have demonstrated that zebrafish are capable of developing a preference for the side opposite to where they previously saw their conspecific images, a response that may be interpreted as sign of temporal anticipation (Pather and Gerlai, 2009). Interestingly, however, when the stimulus was presented at a fixed or at randomly varying locations we found no evidence of preference for either side of the tank. In a recent study we argued that perhaps having to stay in one location of the test environment was too stringent a requirement for the fast moving zebrafish (Jia et al., 2014). However, exactly for this reason a method that employs this requirement may make a learning task more sensitive to detect memory abnormalities, a working hypothesis that led to the current study.

In the current study, we investigate whether zebrafish can remain in one spatial position, i.e. close to a prior constant location of conspecific images in the shuttle box, essentially a passive appetitive task. We utilize the principal design features of the previously employed shuttle box task (Pather and Gerlai, 2009). However, we implement a number of methodological enhancements including computerization of both stimulus delivery and response quantification. Using this new version of the task, we study the effect of two stimulus presentation schedules: delivery of stimuli at fixed versus at random presentation sides. We conduct the experiments in the hope that they will lead to the development of efficient learning and memory quantification paradigms that will allow us to investigate molecular mechanisms of these processes using mutation and/or drug screens in zebrafish.

2. Methods

2.1. Animals and Housing

Seventy zebrafish (approximately 50/50 female/male) of the AB strain that were at least 4 months old were used in the current study. The AB strain was chosen because it is frequently a studied zebrafish strain and it has been used in the analysis of learning and memory too (e.g. Jia et al., 2014). All fish were bred and maintained in the UTM Vivarium (University of Toronto Mississauga, Ontario, Canada) according to CCAC (Canadian Council on Animal Care) standards. The zebrafish were housed in 38 L holding tanks (25 × 50 × 30 cm, width × depth × height) with 18 zebrafish per tank density. Ten percent of the water was replaced with fresh system water (de-ionized oxygenated water supplemented with 60 mg/L Instant Ocean Sea Salt, Big Al's Pet Store, Mississauga, Ontario, Canada) every week in each holding tank. The temperature of the water was held constant at 27 °C by a thermostat. Oxygenation of the water was provided by aeration stones connected via rubber tubing to air compressors. The water was filtered by an overhang power-filter which had mechanical (sponge), biological (bio-wheel) and activated carbon filtration (Marineland Penguin 200). The light cycle was controlled by fluorescent lights on the ceiling which turned on at 9:00 h and off at 23:00 h. The fish were fed flake food (Tetramin Tropical Flakes, Tetra, USA) three times daily at specific times, i.e. at 10:00 h, 15:00 h, and 18:00 h.

2.2. Behavioral testing

The experiment was conducted in an 83-L testing tank (76 × 33 × 33 cm, width × depth × height). The testing tank was illuminated by a fluorescent lamp (Aquarium Spectrum; 15 W and 50 cm long) placed immediately above the tank, which provided 550 lx light intensity as measured inside the test tank at the middle of the water column. Subjects' behavior was recorded by a camera (JVC Everio GZ-MG750) that was placed 185 cm away in front of the tank. A monitor (Acer X193W) was placed on each short side of the tank, and each monitor was connected to a laptop computer (Dell Vostro 1000). The laptops ran a custom software program (GFA) that displayed 6 images of computer-animated zebrafish on the monitors (Qin et al., 2014) (Fig. 1). The movement parameters of these images were set so that they mimic those of naturally swimming zebrafish (Saverino and Gerlai, 2008). All animated fish were of a photograph of a female wild type striped zebrafish. Female shoals have been found to be preferred by both males and females (Ruhl and McRobert, 2005), and thus the female images were used to minimize potential sex differences. Moreover, the animated zebrafish approximated the size (4 cm long) and speed (2.7 cm/s) of the experimental zebrafish. Zebrafish have been shown to respond to such images as if they were live conspecifics (Qin et al., 2014).

2.3. Procedure

At the time of testing, the zebrafish were four months old (sexually mature young adults). Testing was conducted between 10:00 h and 18:00 h. Each zebrafish was tested once individually.

A net, previously submerged in a sterilizing solution (1 mL/L methylene blue) was used to transfer the fish from the holding tank into the testing tank via a beaker filled with system water. Once the zebrafish was placed in the testing tank, the camera was turned on and the GFA program was started.

The experiment ran for a total of 70 min. The first 10 min was the habituation session during which a blank screen was displayed on both monitors. The remaining 60 min was the stimulus presentation session during which the animated images of conspecifics were presented for one-minute intervals (the stimulus intervals) and in between these intervals a blank screen was displayed on both monitors also for 1 min (inter-stimulus intervals: ISIs). The location of the first stimulus presentation, i.e. whether it was displayed on the right or on the left monitors, varied across fish. A group of zebrafish randomly selected from the experimental pool was assigned to the same-side presentation schedule. These zebrafish were always shown conspecific images on the same side of the tank although the side of presentation varied across experimental subjects. These zebrafish could properly identify the location of prior presentation of stimuli during inter-stimulus intervals in two

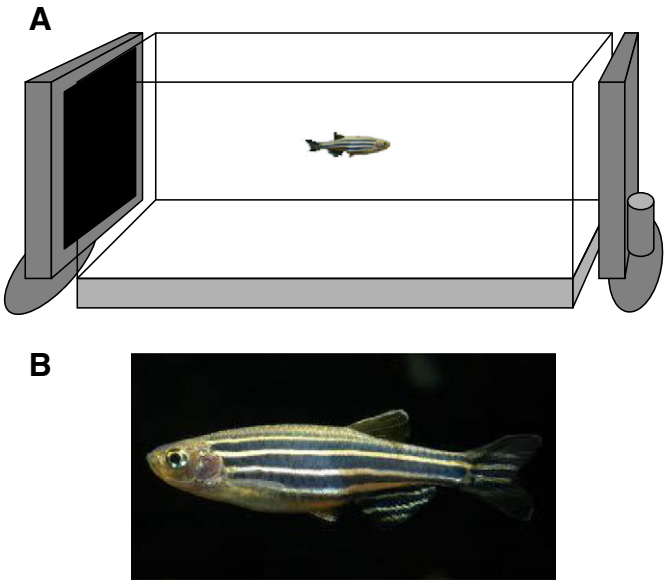

Fig. 1. The experimental set-up (A) and the image of a female conspecific that was used for the animated shoal stimulus (B). Note the two computer monitors (stimulus presentation screens) placed flush against the two side walls of the experimental tank. These monitors could present six animated (moving) images of conspecifics (stimulus) for predetermined periods of time. In one condition, the stimulus was presented repeatedly on the same (fixed) location (either on the left or the right side for the given experimental fish). In the other condition, the side of successive presentations varied randomly.
Modified from Jia et al. (2014) and Pather and Gerlai (2009).